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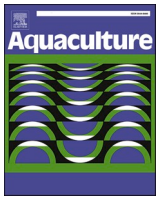

Influence of generations in captivity, life stage and temperature on gene expression in brain tissue of juvenile brook charr (*Salvelinus fontinalis*)

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ABSTRACT

Extended captivity in the stable, low-stress conditions of hatcheries can result in adaptive responses that reduce the phenotypic plasticity of fish species, limiting their ability to respond to environmental changes. This may negatively impact the survival, growth, and behavior of fry when released into natural habitats. Here, we examined differences in the neural transcription of 18 genes involved in four key biological processes (stress response regulation, cellular stress response, appetite regulation, and neurogenesis) in fry and fingerling of brook charr (*Salvelinus fontinalis*) reared at two temperatures (7 °C and 10 °C) and originating from two captive strains of the same genetic origin which differ by the number of generations spent in captivity: the Hill's Lake strain, a long-established captive strain (F > 25), and a recently-established (F1) strain from Scott Lake in Algonquin Park, ON, with substantial Hill's Lake ancestry. The objective was to evaluate whether the transcriptional plasticity of brook charr in response to rearing temperature differences is influenced by domestication across different life stages and to determine whether this plasticity is driven by environmental, genetic, or parental factors. Our findings revealed that multigenerational domestication reduces the threshold for initiating stress response, as evidenced by the upregulation of cellular stress response-related genes in the most domesticated strain. Both groups exhibited similarly low heritability estimates for gene expression variation, indicating a limited long-term adaptation potential under elevated temperature conditions.

1. Introduction

Captive breeding for stocking is increasingly common in salmonid conservation programs to boost population abundance (Johnsson et al., 2014; Ritter, 1997; Létourneau et al., 2018). For species like brook charr (*Salvelinus fontinalis*), this practice is primarily aimed at supporting the recreational fishing industry (Fraser, 2008; Wood, 2017). In both cases, breeders are spawned in captivity, and their progeny are reared in hatcheries before being released into natural habitats. However, differences between hatchery and natural conditions have led to genetic and phenotypic divergence between captive-reared and wild fish (Huntingford, 2004). Rearing fish in artificial environments with no predators, abundant food, and stable conditions may lead to a loss in plasticity due to relaxed natural selection pressures (Hutchings and Fraser, 2008). Previous studies have shown reduced stress sensitivity in captive-reared fish compared to their wild counterparts (Lepage et al., 2000; Verbeek et al., 2008; Douxfils et al., 2011), and extensive differential gene expression between domesticated, newly captive, and wild fish (Douxfils et al., 2011; Wellenreuther et al., 2019).

Non-optimal temperature conditions can initiate a stress response at the brain level, which is primarily mediated by the activation of the hypothalamic-pituitary-interrenal (HPI) axis and the brain sympathetic-chromaffin-cell (BSC) axis (Wendelaar Bonga, 1997; Trenzado et al., 2003; Alfonso et al., 2021). These pathways mediate the release of cortisol and catecholamines, where cortisol, the main stress hormone, regulates several critical physiological and behavioral functions necessary for adapting to stress (Trenzado et al., 2003; Esteban et al., 2004). Cortisol release is triggered by the activation of neuropeptides such as ACTH, CRF, and AVT1 in the brain, with its effects mediated by mineralocorticoid (MR) and glucocorticoid (GR1 and GR2) receptors

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Table 1
Summary of factorial ANOVA for the effects of life stage, generational time in captivity (GTC), rearing temperature (T°), and their two-way interactions on morphometric data (standard length, mass, and Fulton condition factor K).

Effect	df	Mean Squares	F value	*P* value
Standard Length				
Life stage	1	31,388	1521.522	**< 0.001**
GTC	1	58	2.802	0.0946
T°	1	464	22.487	**< 0.001**
Life stage × GTC	1	587	28.447	**< 0.001**
Life stage × T°	1	613	29.723	**< 0.001**
GTC × T°	1	8	0.365	0.5457
Life stage × GTC × T°	1	58	2.798	0.0949
Residuals	627	21		
Mass				
Life stage	1	2825.4	5042.645	**< 0.001**
GTC	1	4.3	7.737	**0.0055**
T°	1	7.9	14.052	**< 0.001**
Life stage × GTC	1	9.8	17.513	**< 0.001**
Life stage × T°	1	15.5	27.736	**< 0.001**
GTC × T°	1	0	0.004	0.9478
Life stage × GTC × T°	1	1	1.734	0.1888
Residuals	627	0.6		
Fulton condition factor (K)*				
Life stage	1	28.869	228.044	**< 0.001**
GTC	1	0.013	0.104	0.7472
T°	1	0.618	4.884	**0.0275**
Life stage × GTC	1	0.149	3.311	0.0693
Life stage × T°	1	0.471	3.723	0.0541
GTC × T°	1	0.007	0.057	0.8122
Life stage × GTC × T°	1	0.011	0.090	0.7649
Residuals	622	0.127		

* Mass data were log-transformed, and Fulton condition factor (K) data were transformed using 1/x transformation.

found in various tissues (Aluru and Vijayan, 2009; Wendelaar Bonga, 1997). The activation of these corticosteroid receptors plays a key role in regulating behavioral responses to environmental changes, particularly by influencing neuroplasticity. Key markers of neuroplasticity include BDNF, PCNA, NEUROD1, NEUROG1, SOX2, and DCX (Sørensen et al., 2013). The relationship between the expression of neurogenesis-related genes and the stress response remains debated. Some studies suggest a negative correlation, with lower gene expression aligning with increased cortisol and stress response-related genes (Colson et al., 2019; Nonnis et al., 2021; Tang et al., 2022), while others report opposite trends (Topal et al., 2021; Johansen et al., 2012). Additionally, at the cellular level, heat shock proteins (HSPs) ensure cellular protection and prevent protein denaturation, further contributing to the stress response (Feder and Hofmann, 1999). Moreover, the regulation of appetite under environmental stress is crucial for managing energy demands, involving signals like CART, AGRP, CCK.L, LEPR, and NPY further influencing the capacity of organisms to cope with stressors (Unniappan et al., 2002; Kehoe and Volkoff, 2007; Forlano and Cone, 2007; Kang et al., 2010; Yan et al., 2011). Thus, investigating the plasticity of key genes involved in stress response regulation, neuroplasticity, cellular stress response, and appetite regulation should efficiently predict how organisms will cope with environmental changes such as those associated with temperature. Despite the potential importance of this, however, the effects on gene expression on transcriptional thermal plasticity in broodstocks that have been held under relaxed selection and artificial (hatchery) conditions for multiple generations remain poorly understood.

Gene-expression plasticity in response to environmental change can have a heritable genetic basis, and the genetic architecture underlying a phenotypic trait (e.g., gene transcription) can potentially be modified by the environment, resulting in genotype-by-environment interactions (G × E interaction) (Nussey et al., 2007). Parental effects are another component that could contribute to trait expression in progeny (Charmantier and Garant, 2005; Visscher et al., 2008). Maternal effects such as maternal provisioning and egg quality are generally detected during early life stages (Heath et al., 1999; Perry et al., 2004), while paternal effects have been far less frequently studied. Still, recent studies revealed the importance of paternal effects on progeny phenotype (e.g., survival, growth, eggs and larval size) (Garant et al., 2003; Green and McCormick, 2005; Fraboulet et al., 2009; Rutkowska et al., 2020). Paternal effects could be significant, particularly in species where fathers provide care (nest building and protection), thus affecting

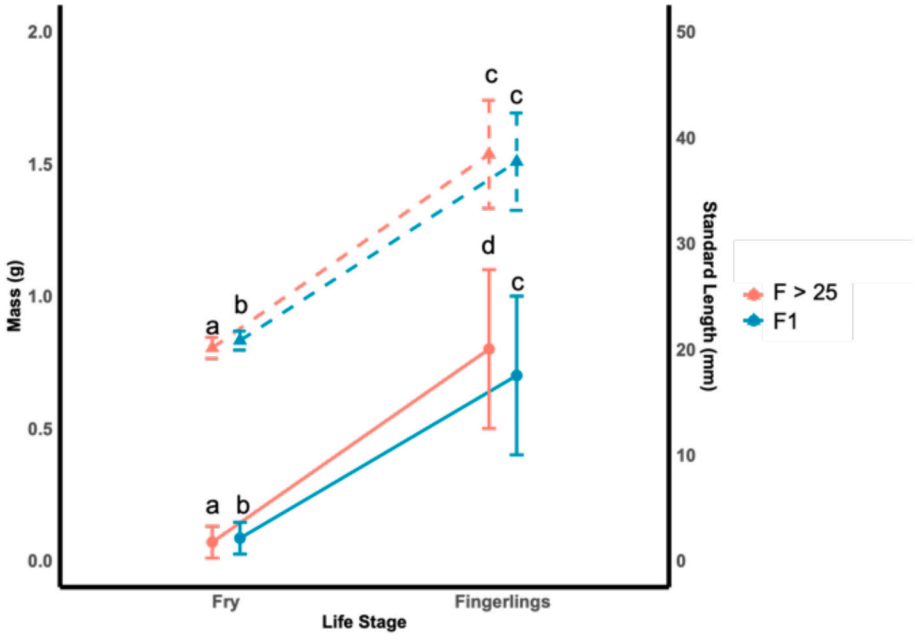

Fig. 1. Growth performance variation (mass and standard length) in both brook charr groups (F1 and F > 25) across two developmental stages (fry and fingerlings). The data presented are prior to any transformation. Individuals from the F > 25 are shown in red, while those from the F1 group are shown in blue. Solid lines represent mass data, and dashed lines represent standard length data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

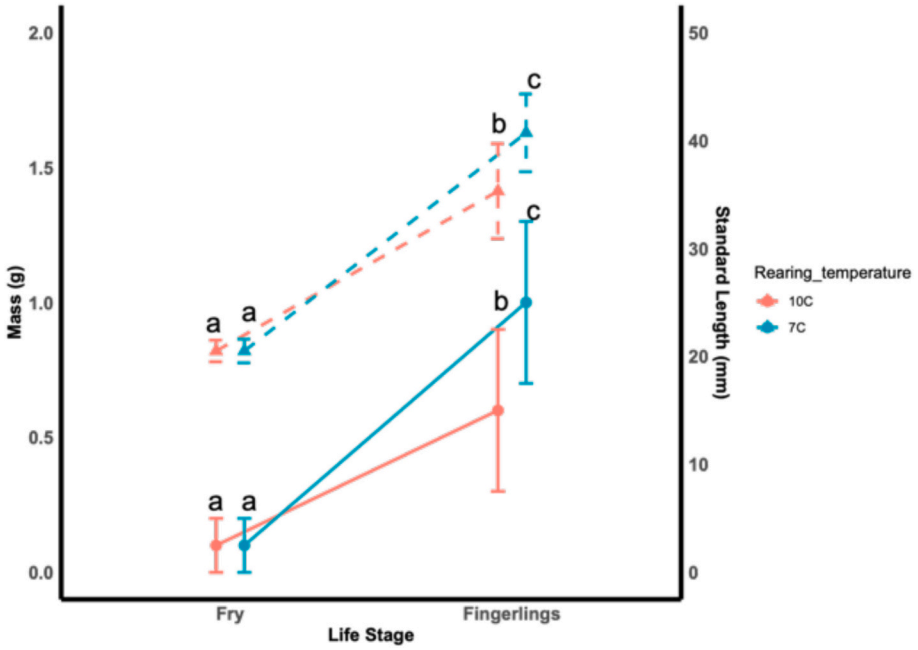

Fig. 2. Growth performance variation (mass and standard length) in the two developmental stages (fry and fingerlings) reared at both temperature conditions (7 °C and 10 °C). The data presented are prior to any transformation. 10 °C is shown in red, while 7 °C is shown in blue. Solid lines represent mass data, and dashed lines represent standard length data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

offspring survival. In species with no paternal care, paternal effects could mainly be mediated through the quality of sperm (Gasparini et al., 2017). It is therefore essential to distinguish genetic effects from environmental and parental influences to better characterize the mechanisms underlying phenotypic plasticity and to predict the long-term adaptation of phenotypic variation in response to environmental changes.

Brook charr (*Salvelinus fontinalis*) is an eurythermal salmonid species that requires cool and well-oxygenated waters. It is native to central and eastern North America ranging from Georgia up to Newfoundland and extending into southern and northern Ontario, Québec, and Labrador (Scott and Crossman, 1973; MacCrimmon and Campbell, 1969). It is a highly prized stocked freshwater species for recreational fishing and is among the earliest (along with lake trout, *S. namaycush*) and most commonly stocked species in Ontario (Wood, 2017; Mitchell et al., 2017). As a thermally sensitive species, there is ample literature on its thermal performance both in captivity and in the wild (e.g., Smith and Ridgway, 2019; Jourdain-Bonneau et al., 2023; Gourtay et al., 2024).

In the present study, we aimed to assess the effect of the number of generations spent in captivity on the expression of brain genes related to stress response regulation (SRR), neuroplasticity (N), cellular stress response (CSR), and appetite regulation (AR) in brook charr fry and fingerlings raised at two different temperatures (7 °C and 10 °C). We hypothesized that progeny from the domesticated Hill's Lake strain (F > 25 generations in captivity) would be less sensitive to temperature variations than progeny from the Scott Lake strain (F1), naturalized population descending from stocked Hill's Lake hatchery fish) due to a loss of phenotypic plasticity. We also estimated heritability and parental contributions to observed phenotypes to better understand sources of phenotypic plasticity. We hypothesized that progeny from the group with the multiple hatchery generations (F > 25) would show minimal heritability estimates in the gene expression variance and that maternal contributions would be more significant than paternal ones in the fry stage compared to fingerling.

2. Materials & methods

2.1. Fish origins, breeding and rearing design

Brook charr breeders used in the present study came from two strains held at the Ontario Ministry of Natural Resources (OMNR) Fisheries Research Facility in Codrington (44.15°N, 77.80°W): the Hill's Lake strain and the Scott Lake strain. The Hill's Lake strain has been used as a provincial broodstock in Ontario hatcheries for nearly a century and more than 25 generations (F > 25 group), with an additional unknown domestication generation in Pennsylvania and is considered heavily domesticated (OMNR, 2005). A single documented wild infusion occurred in 1965–1966 when Hill's Lake females were crossed with F1 males produced from a Hill's Lake × wild Lake Nipigon cross (Fraser, 1981). Since 1980, the Hill's Lake strain broodstock has been maintained using a three-line rotational crossing system to prevent inbreeding. Each generation includes three-year classes, and approximately 50 single-pair crosses are performed per cohort, resulting in a low population-level inbreeding rate (~1/600 per generation). The Scott Lake strain was recently established from a wild spawn collection from Scott Lake in Algonquin Provincial Park, Ontario (45.4856°N, 78.7237°W) for research purposes. The parents of the fish used in this experiment were brought into the facility as wild-origin eggs (~12 families), and were the F1 captive generation (F1 group) (Wilder et al., 2024). It should be noted that Scott Lake was stocked repeatedly using the Hill's Lake strain from 1954 to 1985 (Alshamlih, 2014; Harbicht et al., 2014; OMNR, unpubl. data). Harbicht et al. (2014) showed that most individuals sampled from Scott Lake were admixed, providing clear evidence of heavy introgression from the Hill's Lake hatchery strain. Therefore, the two strains can functionally be considered as differing only in the number of generations being in captivity (Wilder et al., 2024). For both strains, no artificial selection was imposed, and the fish were kept at low density and were fed as much as they needed (ad libitum feeding) to ensure optimal growth and to avoid unintentional selection. Scott Lake fish show growth and maturity patterns similar to Hill's Lake fish but are typically lighter at a given length.

The detailed breeding and experimental designs were described by Wilder et al. (2024), and we used fish at two young stages of

Table 2
Final models retained for each gene. Estimates, p-value, and conditional and marginal r^2. Linear mixed models were used for *AVT1*, *MR*, and *PCNA*. Linear models were used for the other genes. Significance values according to Holm-Bonferroni corrections for each gene are indicated in the tables in parentheses. *AVT1*(Arginine vasotocin), *GR2* (Glucocorticoid receptor 2), *HSD11B2* (11β-hydroxysteroid dehydrogenase 2), *MR* (Mineralocorticoid receptor), *BDNF* (brain-derived neurotrophic factor), *DCX* (Neuronal migration protein doublecortin), *NEUROD1* (Neuronal Differentiation 1), *NGF* (Nerve Growth Factor), *PCNA* (Proliferating cell nuclear antigen), *SOX2* (Transcription factor SOX-2), *CART* (Cocaine and amphetamine regulated transcript protein type II), *CCK.L* (Cholecystokinin), *NPY* (Neuropeptide Y), *CIRBPA* (Cold inducible RNA binding protein), *HSF1* (Heat shock transcription factor 1), *HSP70A* (Heat shock protein 70a), *HSP90AA* (Heat shock protein 90, inducible), *HSP90BA* (Heat shock protein 90, constitutive), GTC (generational time in captivity), T° (rearing temperature).

Genes	Retained models	Variable	Estimates	SE	P-value	Marg. r^2	Cond. r^2
Stress response regulation							
AVT1 (α = 0.013)	Life stage + T° + Generational time in captivity+ Life stage × T° + (1 | sire)	Life stage	0.6254	0.0371	**1.1e-5**	0.410	0.426
		T°	−0.0031	0.0329	0.9249		
		GTC	−0.1387	0.0367	**0.0019**		
		Life stage × T°	−0.1927	0.0525	**0.0002**		
GR2 (α = 0.025)	Life stage + T° + GTC	Life stage	0.0920	0.0113	**2.37e-15**	0.176	0.176
		T°	−0.0428	0.0110	**0.0001**		
		GTC	0.0801	0.0110	**1.27e-12**		
HSD11B2 (α = 0.05)	Life stage + T° + GTC + Life stage × GTC + Life stage × T°	Life stage	−0.0028	0.0056	0.6200	0.048	0.048
		T°	−0.0189	0.0041	**4.71e-06**		
		GTC	0.0025	0.0041	0.5312		
		Life stage × GTC	−0.0182	0.0065	**0.0054**		
		Life stage × T°	0.0245	0.0065	**0.0001**		
MR (α = 0.017)	Life stage + T° + GTC + GTC × T° + (1 | sire)	Life stage	0.2071	0.0130	**1.22e-47**	0.312	0.334
		T°	−0.0152	0.0179	0.3966		
		GTC	0.0961	0.0232	0.0275		
		GTC × T°	−0.0409	0.0255	0.1097		
Neurogenesis							
BDNF (α = 0.025)	Life stage + T° + GTC + Life stage × GTC	Life stage	0.1706	0.0158	**6.17e-25**	0.254	0.254
		T°	−0.0478	0.0109	**1.45e-05**		
		GTC	0.0679	0.0140	**1.66e-06**		
		Life stage × GTC	−0.0481	0.0224	0.0326		
DCX (α = 0.01)	Life stage + T° + GTC + Life stage × T°	Life stage	0.7484	0.0402	**5.04e-62**	0.472	0.472
		T°	0.0678	0.0355	0.0574		
		GTC	0.1427	0.0277	**3.59e-07**		
		Life stage × T°	−0.1880	0.0568	**0.0012**		
NEUROD1(α = 0.008)	Life stage + T° + GTC	Life stage	0.5172	0.0170	**9.09e-126**	0.591	0.591
		T°	0.0115	0.0166	0.4892		
		GTC	0.0175	0.0166	0.2921		
NGF (α = 0.05)	Life stage + T° + GTC + Life stage × T°	Life stage	−3.2235	6.337	0.6111	0.041	0.041
		T°	2.3873	5.606	**0.000299**		
		GTC	2.381	4.374	0.5864		
		Life stage × T°	−2.4386	8.962	**0.003295**		
PCNA (α = 0.017)	Life stage + T° + GTC + Life stage × GTC + Life stage × T° + (1 | sire)	Life stage	0.3276	0.0266	**2.71e-31**	0.384	0.403
		T°	0.2205	0.0192	**1.06e-27**		
		GTC	−0.0129	0.0252	0.6113		
		Life stage × GTC	0.1056	0.0307	**0.0006**		
		Life stage × T°	−0.2625	0.0307	**1.08e-16**		
SOX2 (α = 0.013)	Life stage + T° + GTC + Life stage × GTC + Life stage × T° + GTC × T°	Life stage	0.9156	0.0557	**9.09e-51**	0.511	0.511
		T°	0.0144	0.0506	0.7753		
		GTC	0.3683	0.0513	**1.98e-12**		
		Life stage × GTC	−0.1592	0.0643	0.0136		
		Life stage × T°	−0.1326	0.0643	0.0398		
		GTC × T°	−0.2866	0.0628	**6.07e-06**		
Appetite regulation							
CART (α = 0.017)	Life stage + T° + GTC + Life stage × GTC	Life stage	1.0846	0.0684	**7.06e-48**	0.404	0.404
		T°	−0.3771	0.0473	**7.61e-15**		
		GTC	0.0156	0.0606	0.7962		
		Life stage × GTC	−0.3653	0.0970	**0.0001**		
CCK.L (α = 0.025)	Life stage + T° + GTC + Life stage × T°	Life stage	0.3609	0.0281	**1.40e-33**	0.502	0.502
		T°	−0.2339	0.0249	**1.10e-19**		
		GTC	−0.0873	0.0194	**8.43e-06**		
		Life stage × T°	0.2005	0.0398	**6.33e-07**		
NPY (α = 0.05)	Life stage + T° + GTC + Life stage × T°	Life stage	0.2490	0.0297	**3.79e-16**	0.285	0.285
		T°	−0.1713	0.0263	**1.54e-10**		
		GTC	0.0431	0.0205	**0.0360**		
		Life stage × T°	0.0970	0.0420	**0.0214**		

(*continued on next page*)

Table 2 (*continued*)

Genes	Retained models	Variable	Estimates	SE	P-value	Marg. r^2	Cond. r^2
Cellular stress response							
		Life stage	1.0780	0.0538	**1.67e-69**		
		T°	0.4094	0.0598	**1.89e-11**		
CIRBPA (α = 0.01)	Life stage + T° + GTC + Life stage × T° + GTC × T°	GTC	0.2088	0.0526	**8.10e-05**	0.427	0.427
		Life stage × T°	−0.6668	0.0761	**1.78e-17**		
		GTC × T°	−0.2465	0.0743	**0.0009**		
		Life stage	0.1856	0.0158	**5.83–29**		
HSF1 (α = 0.013)	Life stage + T° + GTC + GTC × T°	T°	0.0184	0.0217	0.3957	0.208	0.208
		GTC	−0.1098	0.0218	**6.62e-07**		
		GTC × T°	−0.0994	0.0308	**0.0013**		
		Life stage	0.7702	0.0754	**9.31e-23**		
		T°	−0.1423	0.0734	0.0529		
HSP70A (α = 0.017)	Life stage + T° + GTC + Life stage × GTC + GTC × T° + (1 | sire)	GTC	0.3445	0.1396	**0.0204**	0.199	0.285
		Life stage × GTC	−0.3263	0.1066	**0.0023**		
		GTC × T°	−0.2650	0.1041	**0.0112**		
		Life stage	−1.1848	0.1207	**2.87e-21**		
HSP90AA (α = 0.025)	Life stage + T° + GTC + Life stage × GTC	T°	0.0309	0.0834	0.7113	0.293	0.293
		GTC	0.3520	0.1070	**0.0011**		
		Life stage × GTC	−0.3664	0.1710	0.0325		
		Life stage	1.0062	0.1060	**4.74e-20**		
HSP90BA (α = 0.05)	Life stage + T° + GTC + Life stage × T°	T°	0.1929	0.0938	0.0501	0.170	0.170
		GTC	−0.2532	0.0732	**0.0006**		
		Life stage × T°	−0.4448	0.1500	**0.0031**		

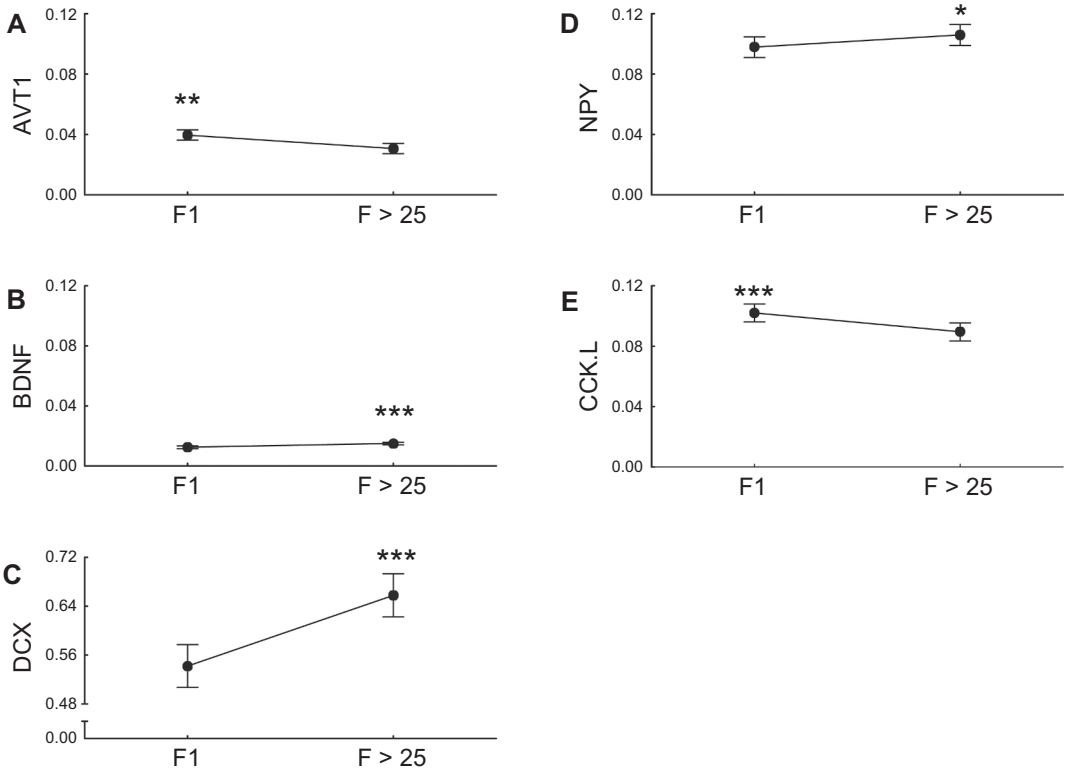

Fig. 3. Relative expression changes of (A) *AVT1* (stress response regulation), (B) *BDNF* and (C) *DCX* (neuroplasticity), and (D) *NPY* and € *CCK.L* (appetite regulation) between individuals from the F1 group and those from the F > 25 group, showing means ± SD. Original data prior to transformation are represented.

development for our study. In brief, for both brook charr groups (F1 and F > 25), spawning was conducted at the Codrington Fisheries Research Facility between mid and the end of November 2019, using ten males and ten females 4-year-old from the same strain (Table S1). Five factorial (2 × 2) crosses were performed for each group. In each cross, eggs from each female were fertilized by two different males and those males were used to fertilize eggs from a second female (Fig. S1). No sibling crosses were performed to prevent inbreeding, and no duplicate crosses were made. In total, ten full-sib and ten half-sib family groups were initially produced for each group. However, because one F > 25 group cross showed poor fry survival, only four crosses per group were ultimately used, resulting in eight full-sib and eight half-sib family groups. After internal disinfection and initial rearing at ambient temperature (5.5 °C to 6 °C), eyed eggs were transported to the Great Lakes Institute for Environmental Research (GLIER) Aquatics Facility at the University of Windsor for rearing. Upon arrival, eggs from each family were divided

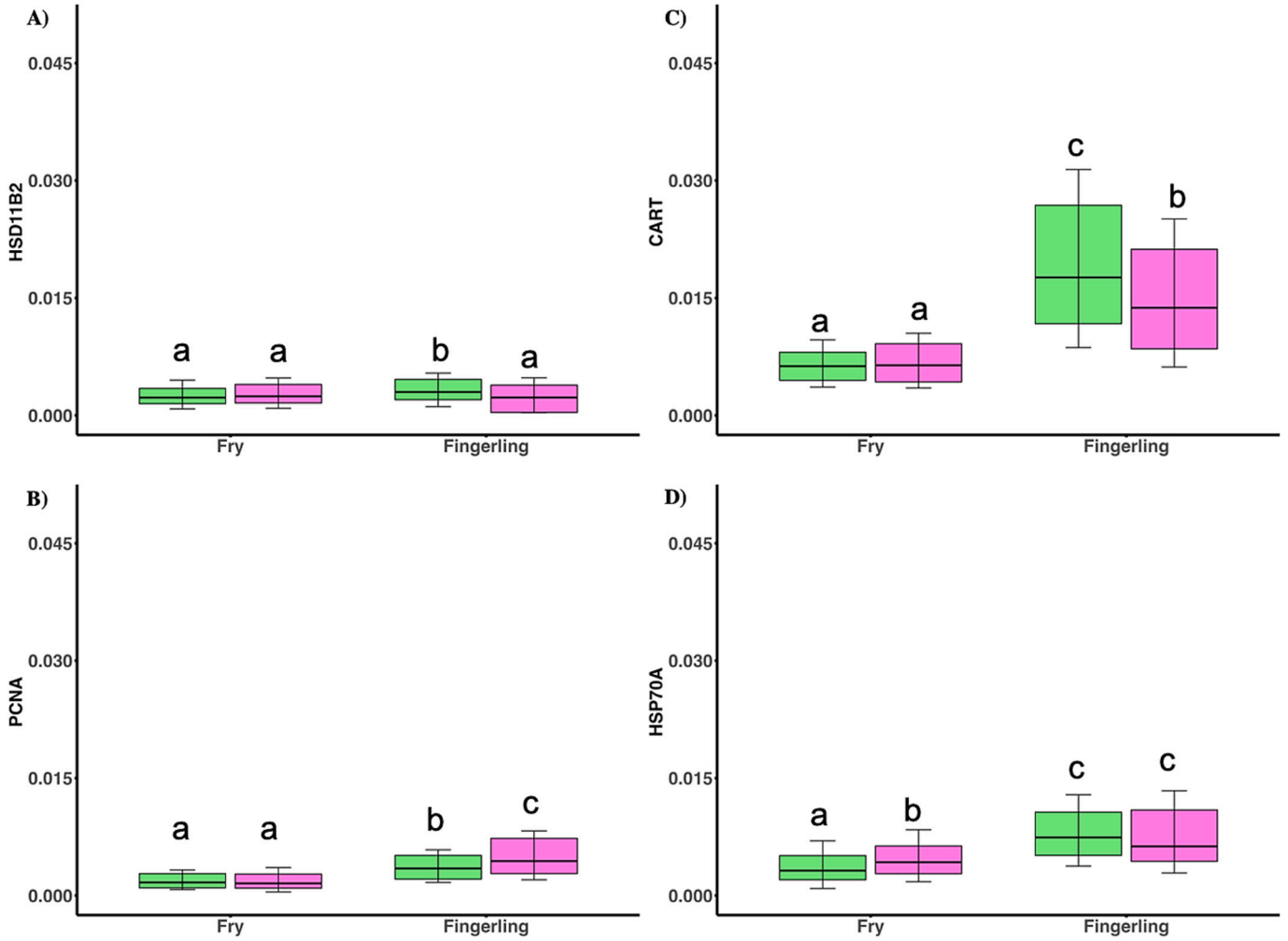

Fig. 4. Box plots of relative expression of (A) *HSD11B2* (stress response regulation), (B) *PCNA* (neuroplasticity), (C) *CART* (appetite regulation), and (D) *HSP70A* (cellular stress response), depending on the interaction of life stage and generational time spent in captivity. F1 group is shown in green and F > 25 group is shown in magenta. Original data prior to transformation are represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

into four replicates per group by equal eggs volume (463 mL ± 79.456): two replicates were reared at 7 °C, similar to the production temperatures at the Codrington hatchery (Hokanson et al., 1973; Howell et al., 2022), and the remaining two replicates were reared at 10 °C, representing the projected +3 °C increase for the region's freshwater ecosystems (Austin and Colman, 2007; Trumpickas et al., 2009; Van Vliet et al., 2013). For the present study, at the yolk sac resorption (fry) stage (10 °C- 934 degree-days [~ 3 months post fertilization]; 7 °C- 961 degree-days [~ 5 months post fertilization]) and after three to four months of exogenous feeding (fingerlings; 1765 degree-days at 10 °C [~ 6 months post fertilization] or 1799 degree-days at 7 °C [~ 9 months post fertilization]), 6 fry and 4 fingerlings per family and per group (F1 and F > 25) were sampled, weighed, and their total and standard lengths recorded. The samples were then stored in 15 mL or 30 mL Falcon tubes filled with RNA-Later at −20 °C until further analysis. Fulton's condition factor (K) was calculated using the mass and total length according to Barnham and Baxter (1998).

2.2. RNA extraction and reverse transcription

In August 2023, the stored samples were transported to the *Institut des Sciences de la Mer de Rimouski (ISMER)* at *Université du Québec à Rimouski* (UQAR) for RNA extraction and cDNA synthesis. Whole brain tissue was dissected for a total of 389 fry from 8 full and half-sib families (6 per family, per temperature treatment, per group) and 250 fingerlings from 8 full and half-sib families (4 per family, per temperature treatment, per group) using a binocular microscope (Leica MZ95) (Table S2). Using the RNeasy Fibrous Tissue Kit (Qiagen. Inc., Mississauga, ON, Canada), total RNA was extracted and then diluted to a final concentration of 200 ng uL^{-1}. RNA purity (ratio A260/280) and integrity were verified using a Nanodrop ND-1000 Spectrophotometer version 3.3.0 (NanoDrop Technologies, Inc., Delaware, USA) and SYBR Safe DNA Gel Stain 2% agarose gel electrophoresis (Alpha Imager HP System, Alpha-Innotech, Alpha Software, Invitrogen, Inc., CA, USA) respectively. All A260/280 ratios were between 1.7 and 2.1. cDNA was obtained from the total RNA using the Quantitect Reverse Transcription Kit (Qiagen, Inc.) and then diluted to a final concentration of 150 ng uL^{-1}. cDNA purity was assessed using the Nanodrop ND-1000 Spectrophotometer version 3.3.0. cDNA samples were sent to GLIER at the University of Windsor for gene transcription quantification using TaqMan OpenArray chips (Applied Biosystems).

2.3. Expression quantification analysis

Gene expression was quantified using a high-throughput nanofluidic OpenArray Stress Transcriptional Profiling Chip (STP-Chip) (GEN-FISH Consortium) for salmonid neural tissue on a Quant-Studio 12 K Flex Real-Time PCR system. Each chip allows simultaneous duplicate

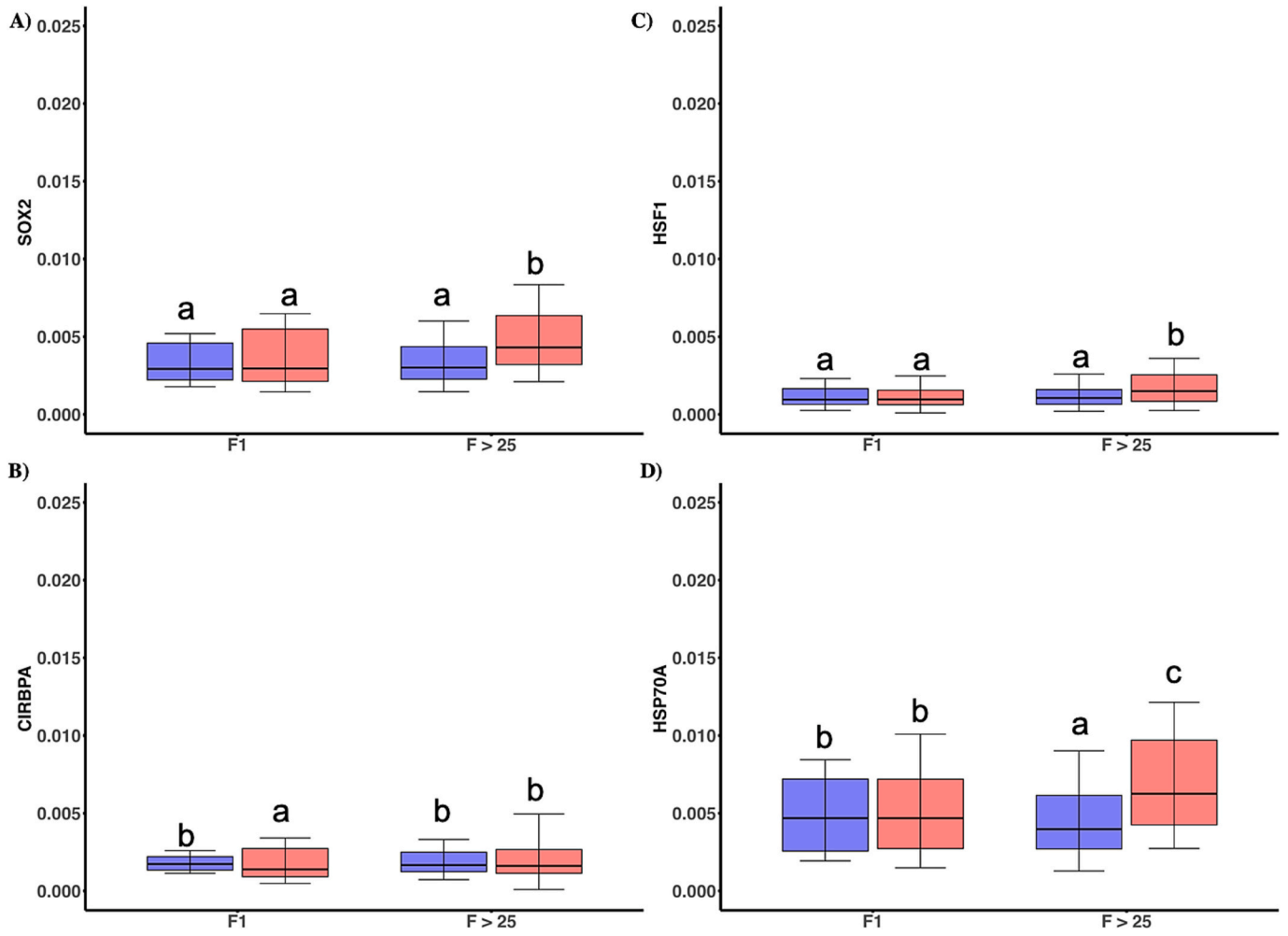

Fig. 5. Box plots of the relative expression of (A) *SOX2* (neuroplasticity), (B) *CIRBPA*, (C) *HSF1*, and (D) *HSP70A* (cellular stress response) depending on the interaction between generational time in captivity and rearing temperatures. Rearing temperature of 7 °C is shown in blue, and rearing temperature of 10 °C is shown in red. Original data prior to transformation are represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysis of 28 genes (24 target genes and 4 reference genes) across 48 samples. A total of 14 chips were used to process 639 samples, with each sample analyzed in duplicate. The 24 target genes are involved in four biological processes related to environmental change response: stress response regulation (*MR, GR1, GR2, HSD11b2, AVT1, CRFB1*), neurogenesis (*BDNF, PCNA, NEUROD1, NEUROG1, DCX, SOX2*, *NGF*), cellular stress response (*HSP70a, HSP90aa, HSP90b, CIRBPA, HSF1*), and appetite regulation (*CCK.L, POMC, NPY, LEPR, AGRP-1, CART*). *EF-1 A* (Elongation factor 1 alpha), *RPL13A* (Ribosomal protein L13a), *RPL7* (60S ribosomal protein L7), and *RPS9* (40S ribosomal protein S9) served as reference genes. Primers and probes sequences were pre-designed by the GEN-FISH team targeting the most conserved regions to be used on various salmonid species including brook charr. Most primer sequences showed 100% homology with brook charr gene sequences, verified in a previous study using the same salmonid brain gene expression chip (Banousse et al., 2025). The primers and probe sequences, along with their efficiency values (calculated using LinRegPCR), are presented in Table S3. Six genes (*GR1, AGRP, CRFB1, NEUROG1, POMC-1, LEPR*) for which expression was very low or not detected were excluded from the analysis.

For each gene and sample, duplicate Crt values were averaged, and the starting concentration of DNA (N_0) was calculated using efficiency values following Tuomi et al. (2010) to correct for PCR efficiency differences. The stability of the combination of the four reference genes (geometric mean) was verified using Normfinder (Andersen et al., 2004). ΔCT values were calculated as the ratio of N_0 to the "reference" value.

2.4. Statistical analysis

For morphometric data (mass, standard length, and Fulton's condition factor), the effect of generational time in captivity, life stage, rearing temperature, and their interactions were analyzed using three-way factorial ANOVA (Statistica, version 6.1.478, Statsoft). Mass and Fulton's condition factor data were log and 1/x transformed respectively before analysis to achieve normality. In the absence of homoscedasticity, a posteriori LSD Fisher tests on ranks were performed ($\alpha = 0.05$).

For each gene, descriptive statistics (sample size, mean, standard deviation, minimum and maximum) were calculated (Table S4). All gene expression data were transformed using Box-Cox transformation to achieve normality using the *preProcess* function in the *caret* package, which estimates the optimal lambda (λ) separately for each gene (V.6.0–94, Kuhn, 2008). Linear Mixed Models (LMM) from the lme4 package (V.1.1–35.3; Bates et al., 2015) were used to evaluate the main effects of life stage, generational time in captivity, rearing temperature, and their interactions (life stage × generational time in captivity × rearing temperature, life stage × rearing temperature, life stage × generational time in captivity, generational time in captivity × rearing

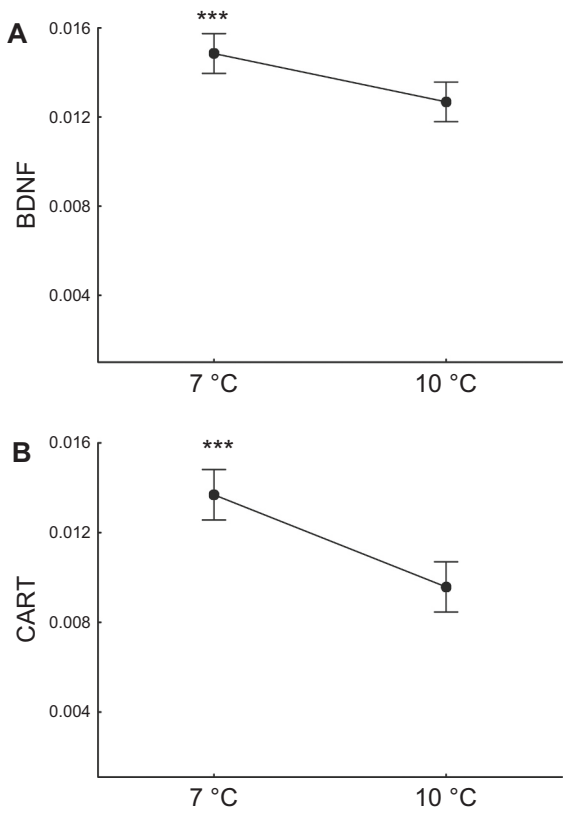

Fig. 6. Relative expression changes of (A) *BDNF* (neuroplasticity), and (B) *CART* (appetite regulation) depending on rearing temperatures (7 °C and 10 °C) shown as mean ± SD. Original data prior to transformation are represented.

temperature) on the expression of 18 target genes (*AVT1, GR2, HSD11B2, MR, BDNF, DCX, NEUROD1, NGF, PCNA, SOX2, CART, CCK.L, NPY, CIRBPA, HSF1, HSP70A, HSP90AA, HSP90BA*). Dam ID, sire ID, family ID, and chip ID were included as random factors in the full model. Model selection for random effects was performed using backward elimination. Reduced models were generated by sequentially removing one random effect at a time from the full model, and each reduced model was compared to the corresponding full model using likelihood ratio tests (LRTs) based on ANOVA comparisons. Random effects that did not significantly improve model fit ($p > 0.05$) were excluded from the final model. The same method was applied for selecting fixed effects in the final models retained. Significant interactions were further analyzed with LSD test using the emmeans package (Lenth et al., 2023). Conditional and marginal R^2 values were calculated using the MuMIn package (V.1.47.5; Barton, 2009). *P*-values were adjusted for each gene using the Bonferroni-Holm method within each functional group.

Reduced models were generated by sequentially removing one random effect at a time from the full model, and each reduced model was compared to the corresponding full model using likelihood ratio tests (LRTs) based on ANOVA comparisons. Random effects that did not significantly improve model fit ($p > 0.05$) were excluded from the final model.

Heritability, maternal, and paternal contributions were estimated separately within each life stage (fry and fingerlings), generational time in captivity (F1 and F > 25 groups), and rearing temperature (7 °C and 10 °C) using restricted maximum likelihood (REML) implemented in ASREML (V4.2 Butler et al., 2023). Our breeding design was partially factorial, with dams and sires both shared among half-sib families. Because this structure generates non-independent genetic relationships, variance components were estimated using animal model, which incorporates the full relationship matrix (pedigree) to partition additive genetic, maternal, paternal, and residual variances. Eight models, incorporating different combinations of random effects, were fitted for each gene. These included models with: (i) additive genetic effects only, (ii) dam effects only, (iii) sire effects only, (iv) additive genetic and dam effects, (v) additive genetic and sire effects, (vi) dam and sire effects, and (vii) additive genetic, dam, and sire effects. A full model further included the dam-by-sire interaction. No additional fixed effects were included, as analyses were conducted separately for each developmental stage, generational time in captivity, and temperature treatment.

The full model can be written as follows:

$$y_{ijk} = \mu + A_i + D_j + S_k + (D \times S)_{jk} + e_{ijk}$$

where y is the phenotypic observation, μ is the overall mean, A_i is the individual random additive genetic effect linked to the pedigree structure, D_j is the random effect of Dam, S_k is the random effect of Sire, $(D \times S)_{jk}$ represents the dam by sire interaction, *and* e_{ijk} *is* the residual effect.

These candidate models are not strictly nested; therefore, likelihood ratio tests were not appropriate. Instead, models were selected based on their AIC (Akaike Information Criterion) weight, and only models with an AIC > 0.10 were retained for variance component calculation according to Vega-Trejo et al. (2018) (Tables S5-S12). Variance components were extracted from these retained models.

Total phenotypic variance (V_P) was calculated as the sum of the variance components estimated in each of the retained models as follows:

$$V_P = V_A + V_M + V_F + V_R$$

where V_A is the additive genetic variance linked to the pedigree structure, V_M is the dam variance, V_F is the sire variance, and V_R is the residual variance. The full model had convergence issues and resulted in having an AIC weight < 0.10 for all the genes, and was therefore not retained. Consequently, variance components associated with this effect were not included in the calculation of the total phenotypic variance. Narrow-sense heritability (h^2) was calculated as the ratio of additive genetic variance to total phenotypic variance ($h^2 = V_A/V_P$). Maternal contributions to phenotypic variance were estimated as the ratio of dam variance to total phenotypic variance (V_M / V_P). Paternal contributions were estimated as the ratio of sire variance to total phenotypic variance (V_F / V_P). The residual variance was similarly calculated.

Linear models were then used to evaluate the effects of life stage, generational time in captivity, and rearing temperature, along with their interactions on heritability estimates, using backward elimination as described earlier. For significant interactions, Tukey's HSD test was conducted, and *p*-values were adjusted using the Bonferroni-Holm correction method within each functional group. All statistical analyses were performed using R software (V. 4.0.5; R Core Team, 2021).

To assess G × E interactions for each generational time in captivity (F1 and F > 25 groups) at the two developmental stages (fry and fingerling) under the two rearing temperatures (10 °C vs. 7 °C), we used linear mixed models (LMMs). We included rearing temperature (environment), family ID (genotype), and their interaction as random effects. The model can be written as follows:

$$y_{ij} = \mu + F_i + T_j + (F \times T)_{ij} + e_{ij}$$

where y_{ij} is gene expression, μ is the intercept, F_i is the random effect of family identity that represents the genotype, T_j is the random effect of rearing temperature that represents the environment, $(F \times T)_{ij}$ is the family-by-temperature interaction term representing genotype-by-environment (G × E) variation in reaction norms, and e_{ij} is the residual error.

All families included in the analyses were represented in both temperature treatments. To test the significance of G × E interactions we compared the full model (with the interaction term) to a reduced model (without the interaction term). The effect size of the G × E interaction was quantified by calculating the proportion of total variance

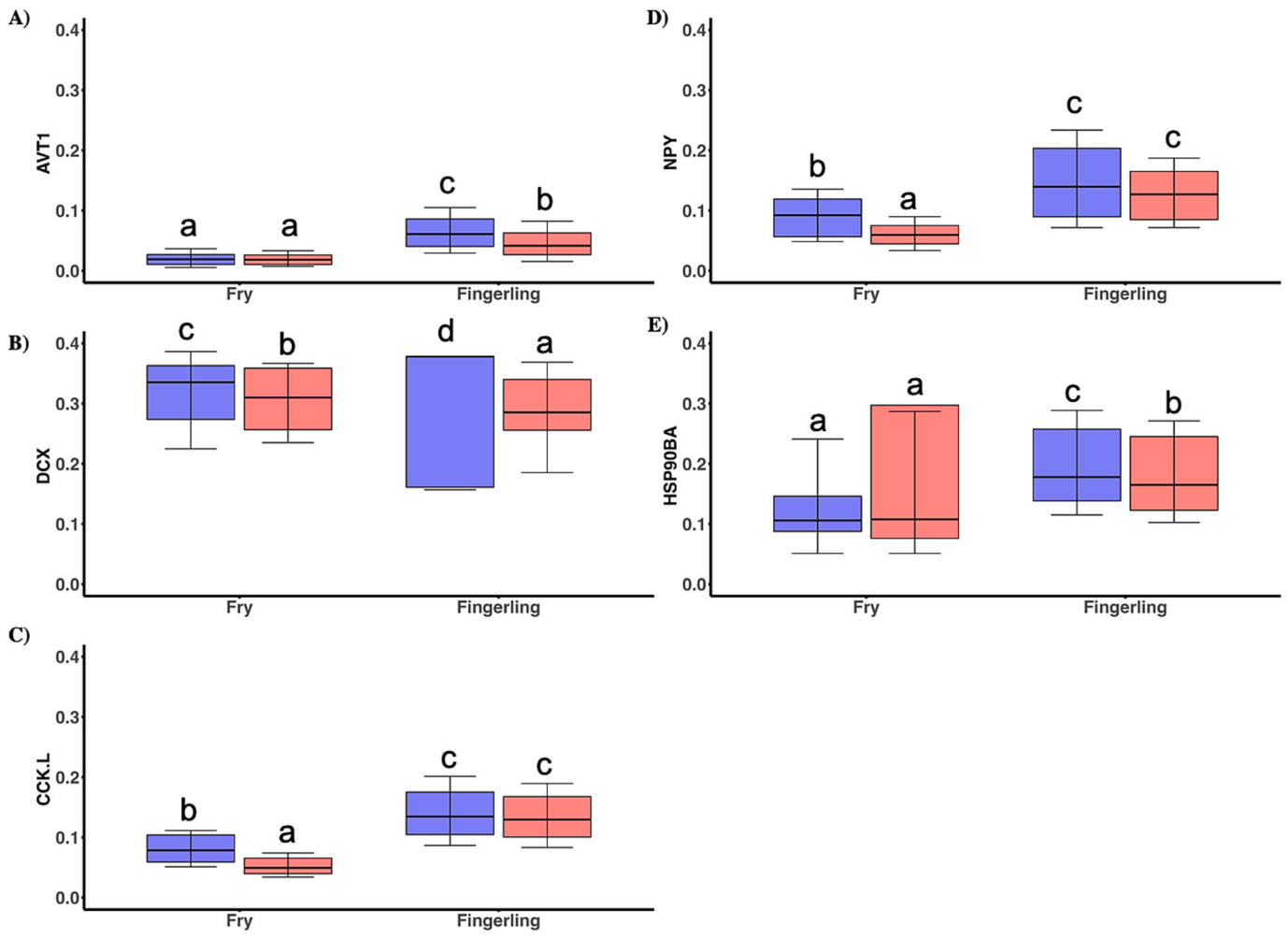

Fig. 7. Box plots of the relative expression changes of (A) *AVT1* (stress response regulation), (B) *DCX* (neuroplasticity), (C) *CCK.L*, (D) *NPY* (appetite regulation), and (E) *HSP90BA* (cellular stress response) depending on the interaction between life stage and rearing temperature. Rearing temperature of 7 °C is shown in blue, and rearing temperature of 10 °C is shown in red. Original data prior to transformation are represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

attributable to the variance component of the interaction term. Reaction norms were visualized using predicted values derived from the full model using the ggeffects package (Lüdecke, 2018). Genetic correlations in the expression of each gene between rearing temperatures were estimated using a bivariate mixed model in ASReml v4.2. Gene expressions at 7 °C and 10 °C were treated as correlated traits, with rearing temperature fitted as a fixed effect and the additive genetic effects were modeled using a variance–covariance matrix and a pedigree-based relationship matrix. Genetic correlations were calculated from the estimated additive genetic variance–covariance components using the following formulas:

$$rG = CovA(7^{\circ}C, 10^{\circ}C)/\sqrt{([VA(7^{\circ}C)\cdot VA(10^{\circ}C)]\ )}$$

where *rG* is the genetic correlation between the trait expressed at 7 °C and 10 °C,$CovA(7^{\circ}C, 10^{\circ}C)$ is the additive genetic covariance of the trait between temperatures, and*VA (7 °C)* and *VA (10 °C)* are the additive genetic variances at 7 °C and 10 °C, respectively.

3. Results

3.1. Effects of generations in captivity, life stage, and rearing temperature on growth

At the fry stage, the F1 individuals were longer and heavier than the F > 25 individuals (life stage × generational time in captivity <0.001 for both, Table 1, Fig. 1). By the fingerling stage, however, the two groups did not differ significantly in standard length, and the F1 individuals weighed less than the F > 25 ones (Fig. 1).

At the fry stage, no temperature effect was observed. However, fingerlings reared at 10 °C showed significantly lower mass and standard length compared to those raised at 7 °C (life stage × rearing temperature < 0.001 for both mass and standard length, Table 1, Fig. 2).

3.2. Effects of generations in captivity, life stage, and rearing temperature on gene expression

Target genes varied considerably in their levels of expression between groups, life stages, and temperatures. Variations in the relative expressions of *GR2*, *HSP90AA*, and *HSP90BA* for generational time in captivity effect (Fig. S2), GR2 for rearing temperature effect (Fig. S3), *HSD11B2*, *NGF*, *PCNA*, and *CIRBPA* for the interaction effect of temperature and life stage (Fig. S4), and finally *HSF1*, *SOX2, and HSP90AA* for life stage effect (Fig. S5) were not considered despite apparent statistical differences, as levels of expression for these genes were not meaningfully significant-close to zero when compared to the expression of reference genes.

Differences in gene expression that were solely associated with generational time in captivity were observed in genes associated with

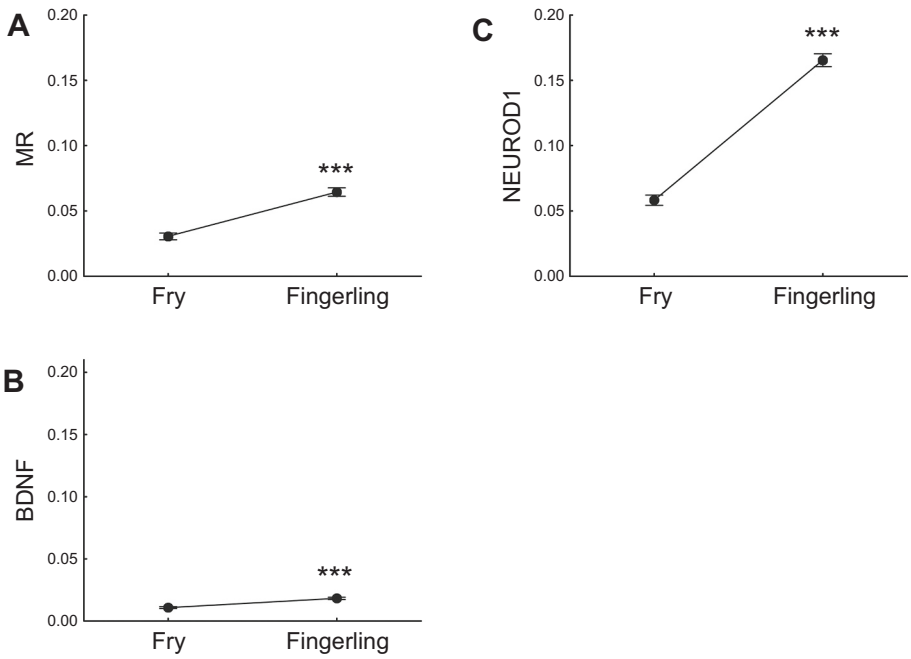

Fig. 8. Relative expression of (A) *MR* (stress response regulation), (B) *BDNF*, and (C) *NEUROD1* (neuroplasticity), across life stages (fry and fingerlings). Mean ± SD. Original data prior to transformation are represented.

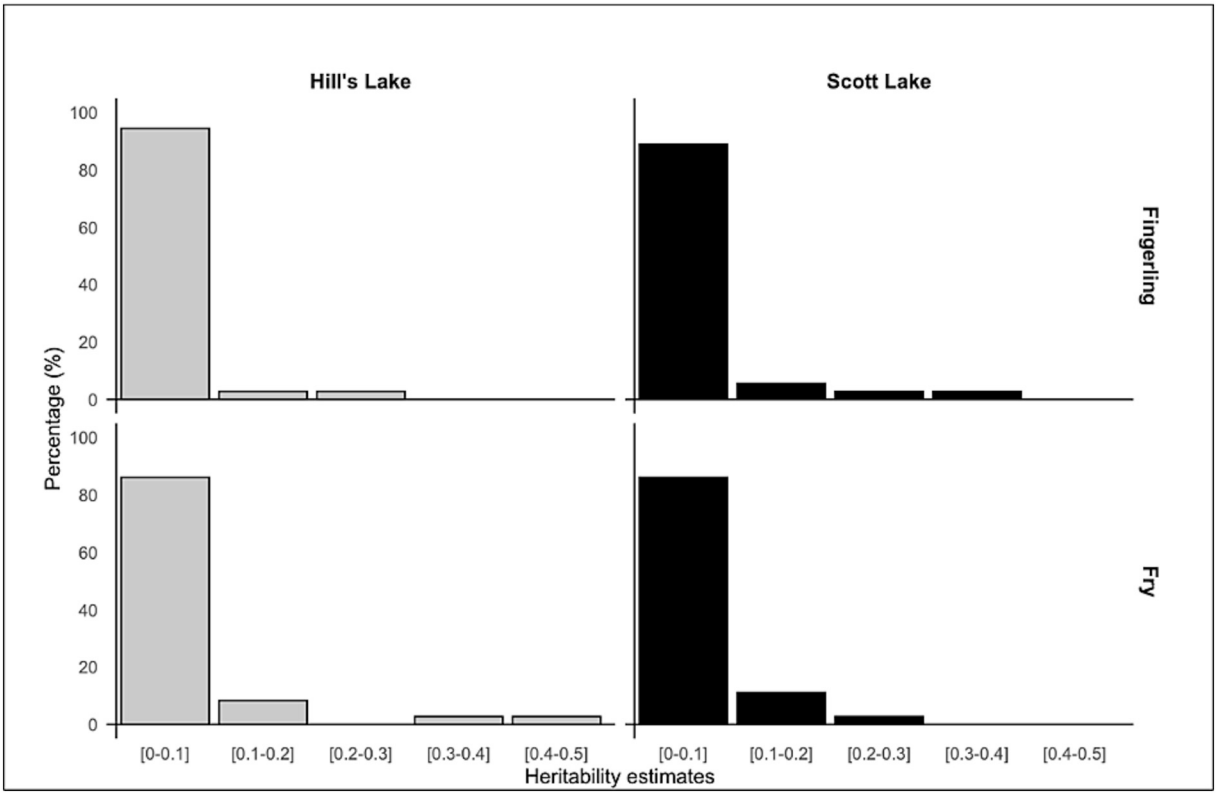

Fig. 9. Histogram showing the distribution of heritability estimates to the total phenotypic variance in the expression of 18 genes in two groups of brook charr (F1 and F > 25) at two developmental stages (fry and fingerling), reared at two different temperatures (7 °C and 10 °C).

the four major functions (Table 2). *AVT1* (stress response regulation [SSR]) was downregulated in individuals issued from the F > 25 group compared to those from the F1 group (Fig. 3A), and *BDNF*, *DCX* (neuroplasticity [N]) and *NPY* (appetite regulation [AR]) were upregulated (Fig. 3B, C, and D). Conversely, *CCK.L* (AR) was downregulated in individuals from the F > 25 group compared to those from the F1 group (Fig. 3E).

The effect of generational time in captivity on gene expression also varied across life stages for a subset of genes linked to the four investigated functions (Table 2). There was no difference in the expression of

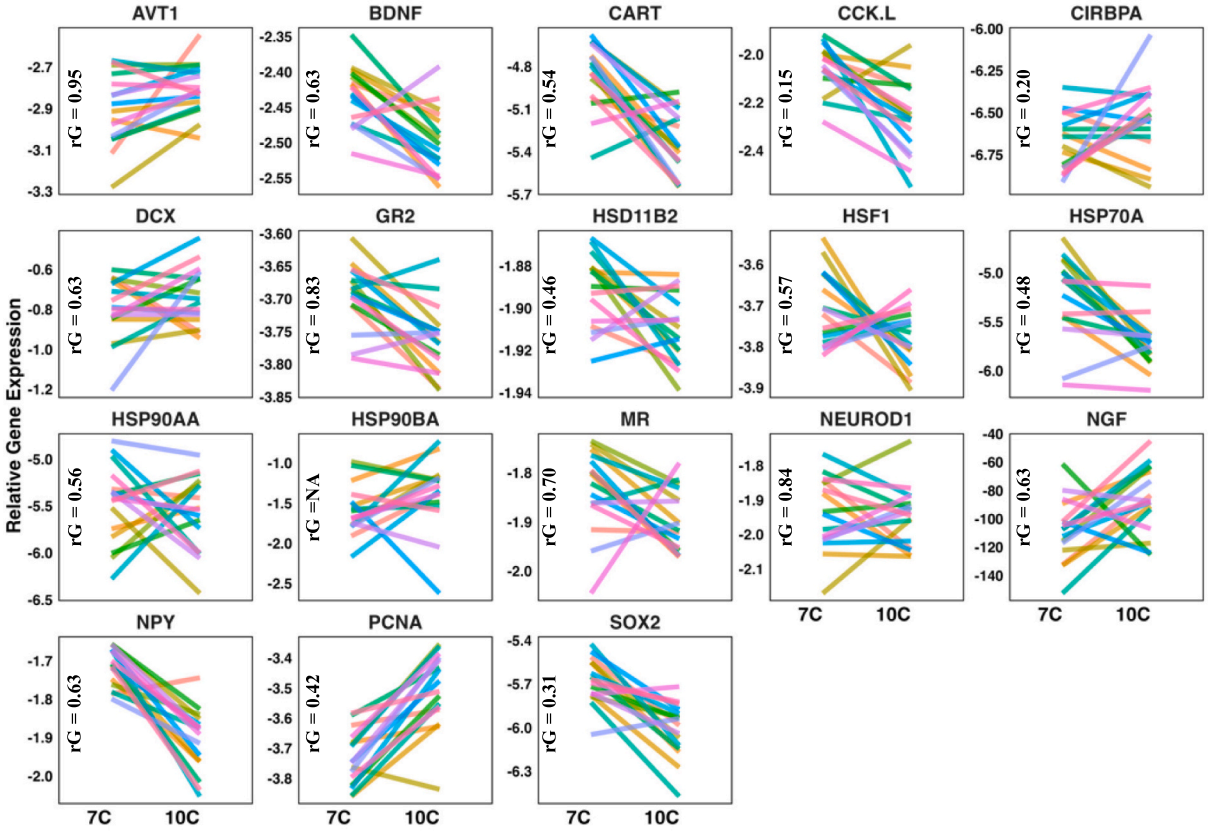

Fig. 10. Reaction norm slopes for the relative gene expression in the brain of brook charr fry from the F > 25 group families (Genotype, $n = 16$) across the two rearing temperature regimes (7 °C and 10 °C) (Environment). Each colored line represents a genotype (family). Genetic correlations (rG) are indicated for each gene. *AVT1*(Arginine vasotocin), *GR2* (Glucocorticoid receptor 2), *HSD11B2* (11β-hydroxysteroid dehydrogenase 2), *MR* (Mineralocorticoid receptor), *BDNF* (brain-derived neurotrophic factor), *DCX* (Neuronal migration protein doublecortin), *NEUROD1* (Neuronal Differentiation 1), *NGF* (Nerve Growth Factor), *PCNA* (Proliferating cell nuclear antigen), *SOX2* (Transcription factor SOX-2), *CART* (Cocaine and amphetamine regulated transcript protein type II), *CCK.L* (Cholecystokinin), *NPY* (Neuropeptide Y), *CIRBPA* (Cold inducible RNA binding protein), *HSF1* (Heat shock transcription factor 1), *HSP70A* (Heat shock protein 70a), *HSP90AA* (Heat shock protein 90, inducible), *HSP90BA* (Heat shock protein 90, constitutive).

HSD11B2 (SRR) at the fry stage, but upregulation was present in the F1 group at the fingerling stage (Fig. 4A). Similarly, *PCNA* (N) expression was similar between the two groups at the fry stage, but upregulation was present at the fingerling stage with a most pronounced increase in the F > 25 group (Fig. 4B). *CART* (AR) also displayed no difference at the fry stage but there was an upregulation in both groups at the fingerling stage, with a larger increase in the individuals from the F1 group (Fig. 4C). At the fry stage, *HSP70A* (CSR) was upregulated in the F > 25 group. We observed an increase at the fingerling stage in both groups, but as the increase was most pronounced in the F1 group, this resulted in similar levels of expression at the fingerling stage (Fig. 4D).

Effects of generational time in captivity × temperature were present in four genes related to neurogenesis and cellular stress response (Table 2). *SOX2* (N), *CIRBPA* (CSR), and *HSF1* (CSR) relative expressions showed no differences between groups at 7 °C but were higher in individuals from the F > 25 group compared to those from the F1 group at 10 °C (Fig. 5A, B, C). *HSP70A* (CSR) was downregulated in individuals from the F > 25 group compared to those from the F1 group at 7 °C and upregulated at 10 °C (Fig. 5D).

3.3. Rearing temperatures effects on gene expression

Only two genes showed expression response relative to temperature: *BDNF* (N) and *CART* (AR) (Table 2) were both downregulated in individuals from both groups raised at 10 °C compared to those at 7 °C (Fig. 6A, B).

Rearing temperature × stage of development effects were present across the four functions (Table 2). *AVT1* (SSR) relative expression increased from fry to the fingerling stage at both rearing temperatures, but decreased at the fingerling stage in individuals reared at 10 °C compared to those reared at 7 °C (Fig. 7A). *DCX* (N) relative expression decreased in fry raised at 10 °C compared to those reared at 7 °C, and increased from the fry to the fingerling stage in individuals reared at 7 °C, while it decreased in those reared at 10 °C (Fig. 7B). At the fry stage, the expression of *CCK.L* and *NPY* (AR) was higher at 7 °C than at 10 °C (Fig. 7C, D). The expression of these two genes increased from the fry to the fingerling stage, but this increase was lower at 7 °C than at 10 °C resulting in final similar levels at this stage of development between the two temperature groups. At the fry stage, HSP90BA (CSR) expression showed no difference between the two rearing temperatures. There was an increase from the fry to the fingerling stage, with a more pronounced response at 7 °C (Fig. 7E).

Regardless of generational time in captivity and rearing temperature, genes associated with SSR, and N showed differential expression between developmental stages. *MR* (SRR), *BDNF* (N), and *NEUROD1* (N) were overexpressed at the fingerling stage compared to the fry stage (Fig. 8A, B, C).

3.4. Genetic basis of the gene expression variance

Overall, the different responses that we observed showed no significant heritability. The average heritability estimate was of 0.04 for the whole sets of genes examined in both brook charr groups (F1 and F > 25), with more than 80% of estimates being below 0.10 (Table S13-S20,

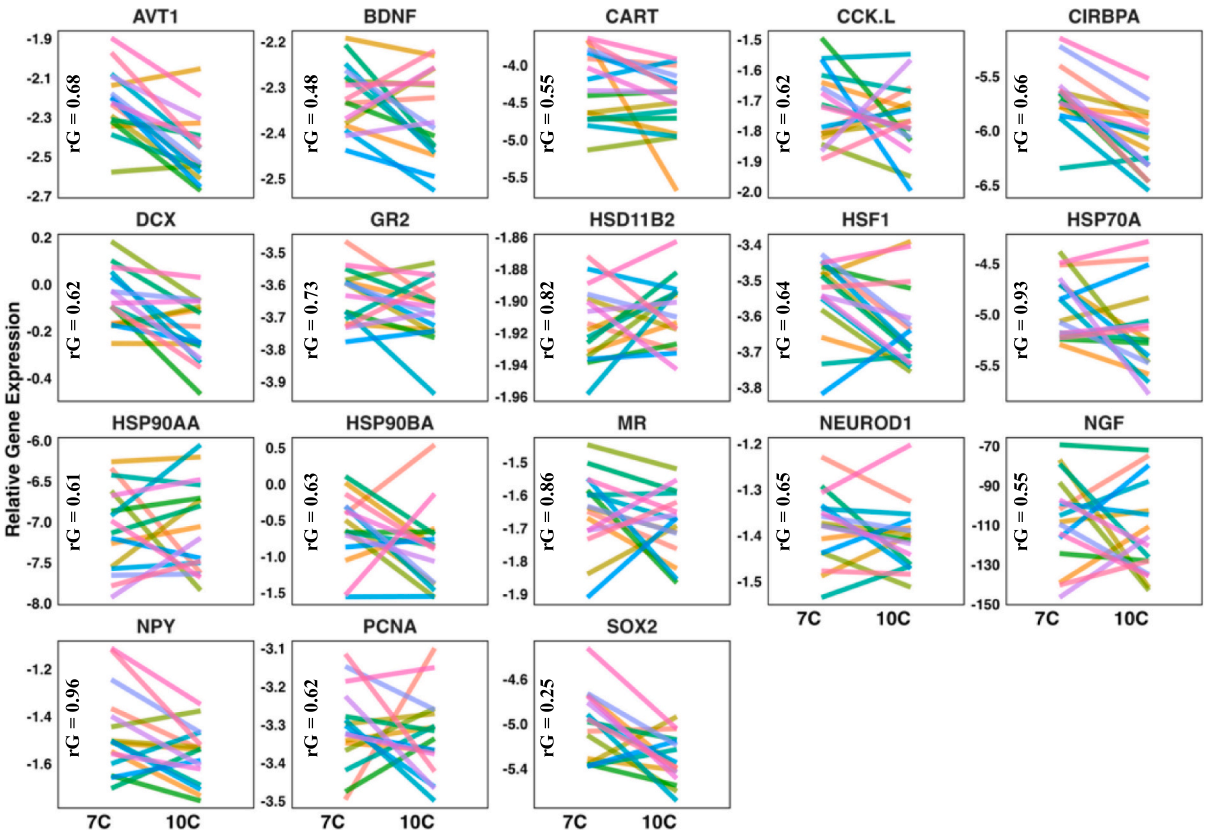

Fig. 11. Reaction norm slopes for the relative gene expression in the brain of brook charr fry from the F1 group families (Genotype, n = 16) across the two rearing temperature regimes (7 °C and 10 °C) (Environment). Each colored line represents a genotype (family). Genetic correlations (rG) are indicated for each gene. *AVT1* (Arginine vasotocin), *GR2* (Glucocorticoid receptor 2), *HSD11B2* (11β-hydroxysteroid dehydrogenase 2), *MR* (Mineralocorticoid receptor), *BDNF* (brain-derived neurotrophic factor), *DCX* (Neuronal migration protein doublecortin), *NEUROD1* (Neuronal Differentiation 1), *NGF* (Nerve Growth Factor), *PCNA* (Proliferating cell nuclear antigen), *SOX2* (Transcription factor SOX-2), *CART* (Cocaine and amphetamine regulated transcript protein type II), *CCK.L* (Cholecystokinin), *NPY* (Neuropeptide Y), *CIRBPA* (Cold inducible RNA binding protein), *HSF1* (Heat shock transcription factor 1), *HSP70A* (Heat shock protein 70a), *HSP90AA* (Heat shock protein 90, inducible), *HSP90BA* (Heat shock protein 90, constitutive).

Fig. 9). No statistically significant effect of generational time in captivity, developmental stage, and rearing temperature on heritability estimates was found (Table S22). Maternal and paternal contributions to gene expression variance were also minimal for both groups (F1 and F > 25) with more than 90% of values being less than 0.10 (average of 0.02) (Tables S13-S20, Figs. S6B, S6C). No statistically significant effect of generational time in captivity, developmental stage, and rearing temperature was found on dam contribution to the expression variance of the 18 genes studied (Table S23). However, the sire contribution to the variance in the expression of *AVT1* was influenced by the interaction between life stage and generational time in captivity, with an increase observed in individuals from the F > 25 group and a decrease in those from the F1 group when reared at elevated temperature (Table S24). Low heritability estimates and parental contributions were also observed when the full dataset (both strains, developmental stages, and rearing temperatures) was analyzed together (Table S21).

No significant genotype by environment interactions was observed in both brook charr groups (F1 and F > 25) or between the two developmental stages in response to different rearing thermal environments (Figs. 10–13, Tables S25-S28). In addition, genetic correlations (rG) for gene expression between 7 °C and 10 °C were predominantly high across most genes with an average of 0.71 for the whole sets of genes examined in both brook charr groups (F1 and F > 25), with more than 80% of estimates being above 0.65 (Figs. 10–13), suggesting minimal genotype-by-temperature interaction.

4. Discussion

The objectives of the present study were to assess the effects of the number of generations spent in captivity on the brain and how they may affect the response to temperature-rearing conditions. On the 18 target genes that we examined, we found differences in expression related to the number of generations time spent in captivity which differed in some instances with temperature conditions and developmental stage. Overall, our results provide partial support for the hypothesis that long-term residence in captivity may alter temperature sensitivity, as individuals from the F > 25 group exhibited shifts in the threshold required to elicit a cellular stress response. However, we were not able to confirm our hypotheses that predicted decreased temperature sensitivity, and a decrease in heritability of expression phenotypes in individuals from the F > 25 group.

Our first hypothesis was that progeny from the F > 25 generation group would be more sensitive to temperature variations than progeny from the F1 group. Individuals from the F > 25 group exhibited higher expression of *HSF1*, *CIRBPA*, and *HSP70A* at the most elevated rearing temperature, these three genes being associated with the CSR function, while no response was observed in the F1 group. This indicates that the number of generations spent in captivity altered the temperature stress response. Specifically, our results indicate that individuals from the F > 25 group may activate CSR genes at a lower or earlier thermal threshold compared with F1 individuals. This observation differs from previous studies, which reported a loss of plasticity in domesticated individuals when exposed to stressors, possibly due to the stable conditions in hatchery environments (Hutchings and Fraser, 2008; Lepage et al.,

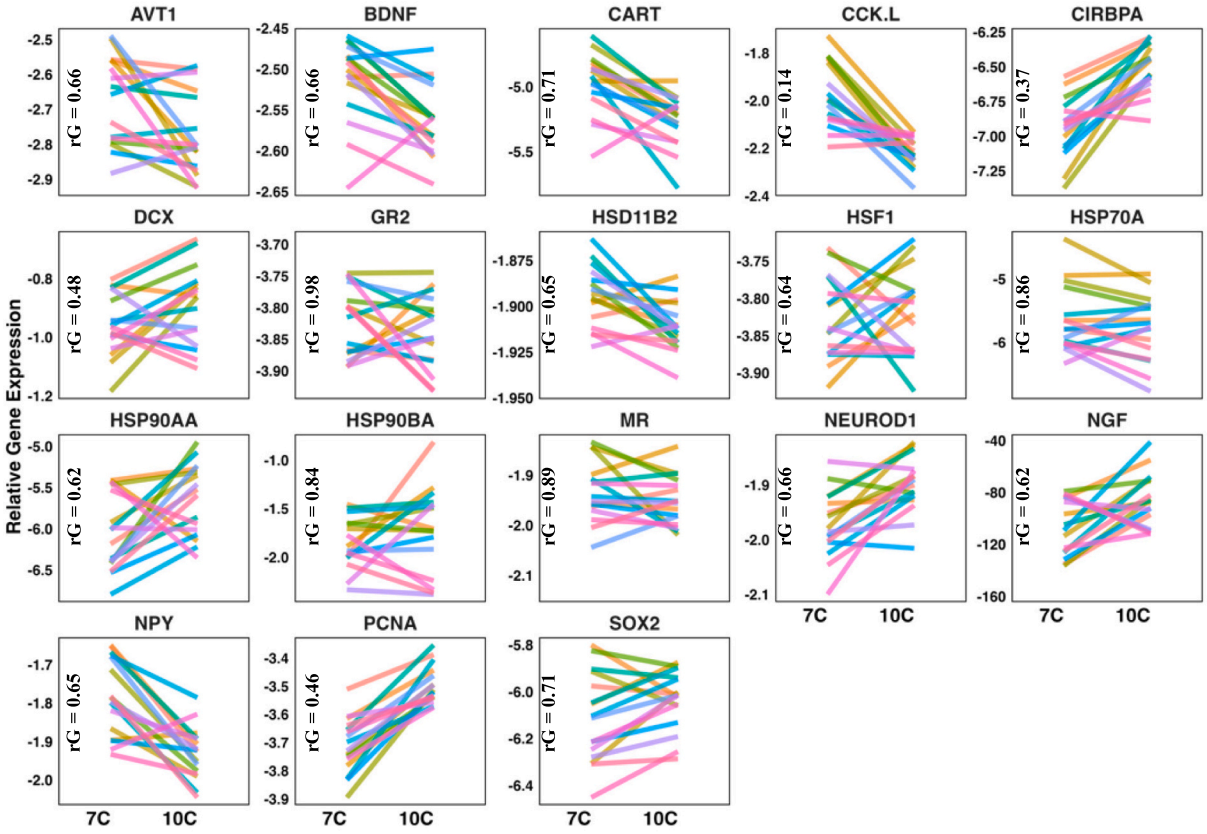

Fig. 12. Reaction norm slopes for the relative gene expression in the brain of brook charr fingerling from the F > 25 group families (Genotype, n = 16) across the two rearing temperature regimes (7 °C and 10 °C) (Environment). Each colored line represents a genotype (family). Genetic correlations (rG) are indicated for each gene. *AVT1*(Arginine vasotocin), *GR2* (Glucocorticoid receptor 2), *HSD11B2* (11β-hydroxysteroid dehydrogenase 2), *MR* (Mineralocorticoid receptor), *BDNF* (brain-derived neurotrophic factor), *DCX* (Neuronal migration protein doublecortin), *NEUROD1* (Neuronal Differentiation 1), *NGF* (Nerve Growth Factor), *PCNA* (Proliferating cell nuclear antigen), *SOX2* (Transcription factor SOX-2), *CART* (Cocaine and amphetamine regulated transcript protein type II), *CCK.L* (Cholecystokinin), *NPY* (Neuropeptide Y), *CIRBPA* (Cold inducible RNA binding protein), *HSF1* (Heat shock transcription factor 1), *HSP70A* (Heat shock protein 70a), *HSP90AA* (Heat shock protein 90, inducible), *HSP90BA* (Heat shock protein 90, constitutive).

2000; Verbeek et al., 2008; Douxfils et al., 2011). However, our results support Stitt et al. (2014) findings which indicated higher *HSP70A* expression in Hill's Lake brook charr submitted to high temperature when compared to northern strains of this species. However, the absence of response in the F1 individuals is difficult to explain. It should be noticed that Wilder et al. (2024) who worked on the individuals from the same experimental design, did not see any differences in terms of survival between emergent fry (fingerling survival differences were not assessed); however, they did find F1 Scott Lake fish experienced a significant increase in their likelihood of morphological malformations from current to elevated temperatures.

We also hypothesized that progeny from the F > 25 group would show reduced heritability estimates in the gene expression variance relative to the F1 group. Again, contrary to our expectations, we found no significant difference in the heritability estimates between the two brook charr groups for the 18 genes examined. This was surprising given the relatively high number of generations the F < 25 group have spent in captivity, and thus have been subject to artificial selection that is consistent in strength and direction contrary to natural selection (Grant and Grant, 2002; Seamons et al., 2007; Carlson and Seamons, 2008). However, these findings align with the shared genetic background of the two groups, which can be attributed to multiple stocking events of the Hill's Lake strain (F > 25 group) into Scott Lake (OMNR, unpubl. data). Our results suggest that the extensive generations in captivity did not significantly influence artificial selective pressures on gene expression. Furthermore, since the Scott Lake strain (F1 group) has not been supplemented with the Hill's Lake strain (F > 25 group) for over ten generations (OMNR, unpubl. data), it indicates that even natural recruitment has not impacted the heritability of gene transcription.

4.1. *Effect of the generational time spent in captivity*

Captivity caused modifications in the expression of genes related to the four functions examined. *DCX*, *BDNF*, and *PCNA* (N) were overexpressed in F > 25 individuals with differences being only observed at the fingerling stage for *PCNA*. Gene upregulation could suggest enhanced neural activation, critical for cognitive abilities such as learning and memory. This contrasts with previous work on fish domestication, which showed downregulation of synaptic functions and neuroplasticity in domesticated Atlantic salmon (Bicskei et al., 2014) and rainbow trout (Tymchuk et al., 2009). However, increased *SOX2* expression in F > 25 individuals reared at 10 °C may represent a downregulation at the protein level, as *SOX2* negatively regulates neurogenesis by preventing neural cells from differentiating into functional neurons (Schmidt et al., 2013). Further studies would be needed to evaluate this global response at the highest temperature. For example, contrasting results were obtained in rainbow trout, with a higher degree of neuroplasticity being associated with high-stress responsive individuals in response to confinement stress (Johansen et al., 2012).

We also observed a reduced expression of *AVT1* and *HSD11B2* (SRR) which both code for proteins involved in cortisol regulation (Wendelaar Bonga, 1997) in individuals from the F > 25 group which suggest adaptation to the hatchery environment. The attenuation of the stress response axis which may enhance productivity-related traits is a primary

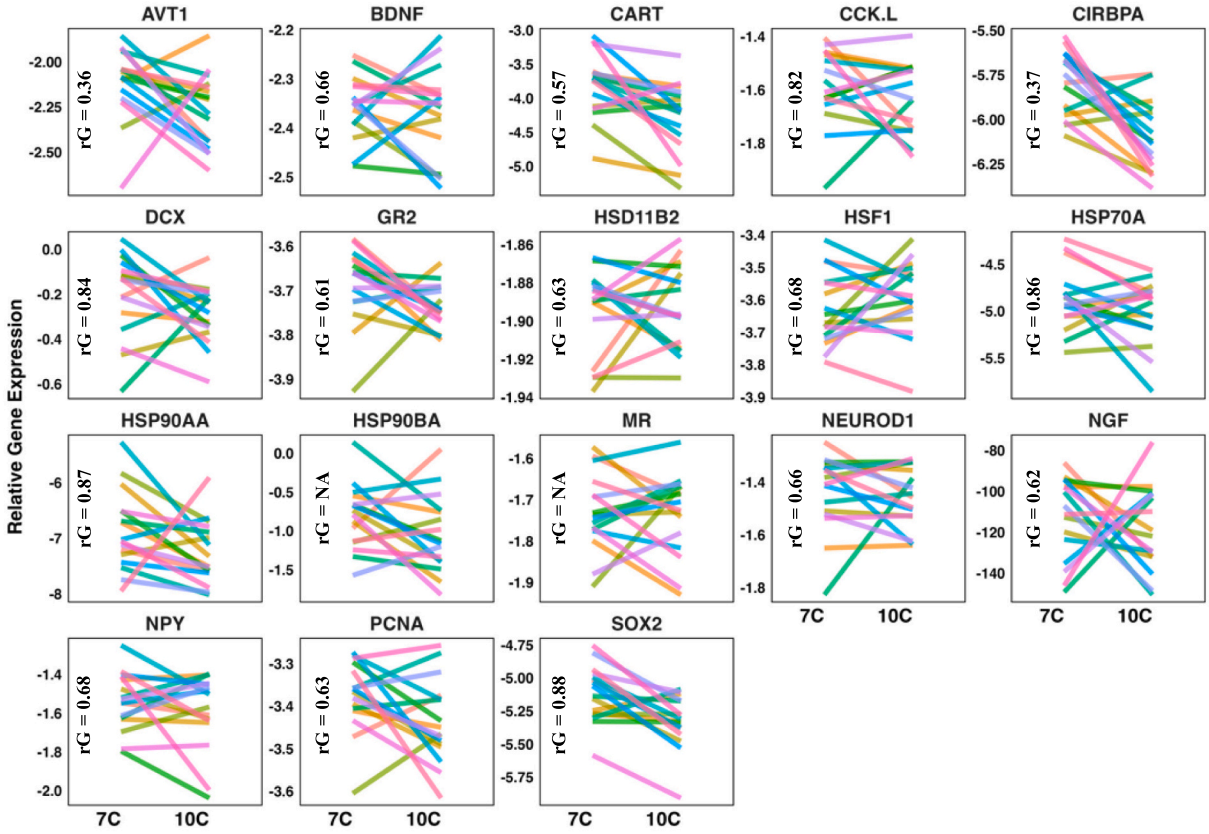

Fig. 13. Reaction norm slopes for the relative gene expression in the brain of brook charr fingerling from the F1 group families (Genotype, n = 16) across the two rearing temperature regimes (7 °C and 10 °C) (Environment). Each colored line represents a genotype (family). Genetic correlations (rG) are indicated for each gene. *AVT1*(Arginine vasotocin), *GR2* (Glucocorticoid receptor 2), *HSD11B2* (11β-hydroxysteroid dehydrogenase 2), *MR* (Mineralocorticoid receptor), *BDNF* (brain-derived neurotrophic factor), *DCX* (Neuronal migration protein doublecortin), *NEUROD1* (Neuronal Differentiation 1), *NGF* (Nerve Growth Factor), *PCNA* (Proliferating cell nuclear antigen), *SOX2* (Transcription factor SOX-2), *CART* (Cocaine and amphetamine regulated transcript protein type II), *CCK.L* (Cholecystokinin), *NPY* (Neuropeptide Y), *CIRBPA* (Cold inducible RNA binding protein), *HSF1* (Heat shock transcription factor 1), *HSP70A* (Heat shock protein 70a), *HSP90AA* (Heat shock protein 90, inducible), *HSP90BA* (Heat shock protein 90, constitutive).

objective of the domestication process (e.g., Lu et al., 2022). However, domestication increased expression of *HSP70A* (CSR) at the fry stage. This gene encodes for a molecular chaperone essential for protecting cells from stress-induced damage (Feder and Hofmann, 1999). In contrast, lower *HSP70A* expression was reported in a comparison between fourth and first generation in captivity in pikeperch (*Sander lucioperca*) (Nynca et al., 2020) and Eurasian perch (*Perca fluviatilis*) (Douxfils et al., 2011). Appetite regulation-related genes were significantly upregulated (*NPY* – appetite stimulator) or downregulated (*CCK.L* and *CART* – appetite inhibitor) in individuals originating from the domesticated group (only at the fingerling stage for *CART*). This may favor an increase in food consumption associated with feeding procedures used in hatcheries. A comparison of the brain transcriptome between farmed and wild Norwegian Atlantic salmon fry at the first exogenous feeding stage revealed an upregulation in several functions related to digestive processes, which could be associated with enhanced food intake efficiency (Bicskei et al., 2014). Our findings also align with the higher mass we observed in fingerlings from the F > 25 group. Many studies showed that the hatchery environment tends to favor individuals with rapid growth rates (Fleming et al., 2002; McGinnity et al., 2003; Solberg et al., 2013; Bellinger et al., 2014).

4.2. Effect of rearing temperatures

Temperature influenced gene expression unrelated to the number of generations spent in captivity. Most of the genes studied showed downregulation at 10 °C compared to 7 °C: *BDNF* and *DCX* (N), *AVT1* (SRR), *HSP90BA* (CSR), and *NPY*, *CCKL*, and *CART* (AR), with a subset of genes altering their expression only at one of the two stages of development. In a previous study on brook charr, examining the whole brain transcriptome of fry, we showed significant downregulation of neurogenesis-related processes in fry originating from warm parents (Banousse et al., 2024). Similar observations have been reported in Atlantic salmon post-smolts exposed to elevated temperatures (16 °C and 18 °C) that exhibited downregulation of *BDNF* and *NEUROD* (Tang et al., 2022), and in rainbow trout fry from females exposed to elevated temperatures (neural and synaptic activity-related processes) (Colson et al., 2019). Such reductions in neurogenesis genes may impair neural functions and, consequently, cognitive abilities. Identifying behavioral patterns and correlating them with gene expression profiles may provide more insight into how altered neurogenesis-related genes can affect key behaviors that are critical for the survival of brook charr during early life stages. The downregulation of *AVT1* and H*SP90BA* is more counter-intuitive. The lower growth performance (mass and length) in fingerlings raised at 10 °C is suggestive of negative temperature impacts. Responses in terms of appetite indicators cannot really inform us, as the expression of both appetite stimulator and inhibitor genes were modified in the same direction. Additionally, individuals were sampled when they reached the same relative developmental stage (fry at first exogenous feeding and fingerlings), based on degree-days rather than time. It is possible that the growth differences observed at the fingerling stage were more related to age than to temperature.

4.3. The genetic basis and parental contributions in the gene expression variance

Using a pedigree-based analysis, we showed that the average narrow sense heritability for gene expression variance was very low for both brook charr groups with different generational time in captivity (h^2 = 0.04). This estimate was comparable to heritability levels that we previously determined in the brook charr Laval strain for the same examined genes (h^2 ranged from 0.03 to 0.07) (Banousse et al., 2025). However, heritability estimates in brook charr were relatively lower than what has been reported in three spine sticklebacks *Gasterosteus aculeatus* (h^2 = 0.23) (Leder et al., 2015), rainbowfish *Melanotaenia duboulayi* (h^2 = 0.25) (McCairns et al., 2016), Chinook salmon *Oncorhynchus tshawytscha* (wild strain, h^2 = 0.14; farmed strain, h^2 = 0.43) (Toews et al., 2019), and Atlantic salmon (Lahave strain (F4), h^2 = 16.7%; Sebago strain (F2), h^2 = 6,0.2%) (He et al., 2017), and could be due to species-specific differences. The observed differences may also be attributed to differences in the specific genes analyzed. While Banousse et al. (2025) examined the same genes as this study, other studies did not, likely reflecting differential selection pressures acting on genes with distinct functional roles (Lynch and Walsh, 1998). For instance, Toews et al. (2019) assessed the heritability of two genes similar to those in our study (*GR2* and *HSP70A*) and reported low heritability for *HSP70A* but moderate heritability for *GR2* in response to immune stimuli in the liver tissue of chinook salmon. Bougas et al. (2010) found that 94% of transcripts in whole fry of brook charr exhibited significant additive genetic variance, including two genes studied here (*HSP90BA* and *CIRBPA*). Heritability estimates for the same genes may vary depending on the tissues examined as well (Price et al., 2011; GTEx Consortium, 2017). To our knowledge, this is one of the first studies in fish to explore the quantitative genetic basis of gene expression specifically at the brain level.

The maternal and paternal contributions to the expression variance of all 18 genes were found to be minimal in both groups of brook charr (0.02) and across the two developmental stages. This finding contrasts with our initial expectations that maternal influences would be more significant in fry than in fingerlings. No difference in maternal contribution to gene expression was similarly found in brook charr fry from the Laval strain (Banousse et al., 2025), and aligns with earlier observations in brook charr for other phenotypic traits, such as survival, standard length, and body mass, examined after yolk sac resorption (Perry et al., 2004; Varian and Nichols, 2010; Crespel et al., 2013; Gourtay et al., 2024), suggesting the absence of significant maternal contributions at these developmental stages.

We did not observe any significant G × E interaction effects on the expression of the 18 genes we analyzed across both groups and developmental stages, suggesting parallel reaction norms. This aligns with our previous findings on brook charr fry from the Laval strain, where we used only full-sub families (n = 8) as a proxy for the genotype (Banousse et al., 2025). In the present study, we also performed the analysis using only full-sib families, and no significant G × E interactions were detected. However, the number of full-sib families per temperature treatment was limited (n = 8). McCairns et al. (2016) demonstrated significant G × E interactions in 9 out of 12 genes when using 19 full-sub families in response to two different temperatures in rainbowfish. This highlights the need for future studies with a larger number of full-sib families per group to confirm our findings.

We did not include the results of nine genes that were barely expressed (Fig. S1, S2, S3, and S4). This does not mean that their expression was not meaningful, but that the very low intensity of the signal precluded any sound interpretation. We used whole-brain RNA, but some genes are expressed in very specific areas of the brain with possible differences in expression being linked with developmental stage. As a result, this specific RNA could be diluted in the whole RNA extract. It is also possible that expression of these genes in other tissues might be more informative.

5. Conclusion

There is ongoing concern about the effects of domestication on fish responses to elevated temperatures associated with climate change. Our findings showed that extended generations in captivity did not reduce plasticity in gene expression variation in response to elevated temperatures. Specifically, individuals from F > 25 exhibited higher levels of expression in cellular stress response-related genes when reared at 10 °C, indicating a lower threshold for initiating stress responses compared with F1 individuals. Nonetheless, it seems that prolonged generations in captivity may lead to an upregulation in the expression of one gene involved in neurogenesis inhibition (SOX2) at 10 °C. Both fry and fingerlings exhibited plastic responses to rearing temperature and domestication across the four examined functions, with gene expression levels increasing from fry to the fingerling stage. Additionally, our results indicated no significant effect of generational time in captivity on heritability estimates for the expression of the 18 genes examined, with both groups displaying similarly low heritability, further challenging our initial expectations. These minimal heritability estimates suggest that while plasticity in gene expression may provide a rapid response to cope with temperature variations that could result in acclimation, long-term adaptation, and evolutionary resilience may still limited. Hatchery management strategies should focus on increasing or maintaining genetic diversity and incorporating environmental enrichment that closely mimics natural conditions to promote neurogenesis under varying environments.

CRediT authorship contribution statement

Ghizlane Banousse: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Céline Audet:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Angélina Lagneau:** Writing – review & editing, Software, Methodology. **Alex Wilder:** Writing – review & editing, Methodology, Data curation. **Chris C. Wilson:** Writing – review & editing, Methodology, Conceptualization. **Christina A.D. Semeniuk:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethics

Animal husbandry, reproduction, and fry rearing were conducted following the recommendations of the Canadian Council on Animal Care. Protocols were performed under the University of Windsor's AUPP 18–08.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.744124.

Data availability

Raw data and all R scripts used in the present manuscript are available in Figshare at https://figshare.com/s/f4a0762e23b43f7085af.

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